Mechanism of Listeria monocytogenes biofilm development and structure analysis of natural flora in food processing environment

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博士学位論文内容要約
Abstract

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論文題目 Title	Mechanism of *Listeria monocytogenes* biofilm development and structure analysis of natural flora in food processing environment(リステリア菌のバイオフィルム形成メカニズムと食品製造現場における微生物フローラの構造解析に関する研究)		

The incidence of foodborne disease outbreaks is increasing globally, leading to many product recalls and critical health problems worldwide. Microbial contamination in food can occur at any point along production line and the most important cause is poor sanitization in processing line. Residual microorganism could attach on the machine surfaces, then strongly persist and survive for an extended period in the structure known as “Biofilm”. The biofilms naturally formed in food processing plants may include various pathogenic, non-pathogenic, and spoilage microorganisms supporting each other to persist and resist environmental stresses. Therefore, understanding the mechanism related to biofilm formation, and also assessment of the diversity and composition of bacterial microflora community existing in processing environment are considered important for designing the effective biofilm control strategies.

Considering the number of food poisoning outbreaks occurred, *Listeria monocytogenes* appears to causes invasive, often fatal infections comparing to other pathogens. Besides, this organism is known to persist in food processing environments and on equipments, sometimes for several years. Therefore, the studies in chapter 2 focused on monoculture bioflim formation of *L. monocytogenes*. In section 1 of chapter 2, the ability of *L. monocytogenes* to form biofilms on conveyor belt surfaces was assessed and the efficiency of cleaning protocol used in food processing plant for eliminating *L. monocytogenes* biofilms was evaluated. This study demonstrates that conveyor belt surface roughness had a great influence on the ability of *L. monocytogenes* to form biofilms, and even more on the susceptibility of these microorganisms to cleaning agents and disinfectants. Thus, to reduce risk of contamination, choosing the optimal belt and performing consistent maintenance are vital for an efficient and hygienic production line.

In section 2 of chapter 2, the change of genes and protein expression level of *L. monocytogenes* at each stage of biofilm development were investigated using RT-PCR and 2D-gel electrophoresis, since it is widely believed that transition from a planktonic to a biofilm mode of growth involves a number of changes in gene regulation. Ten genes including *agrA, agr B, agrD, flaA, prfA, degU, recA, motB, yneA* and *relA* were investigated. The gene that active in early stage of *L. monocytogenes* biofilm formation was *flaA*, whereas the gene that active in late stage were *prfA and recA*. The gene showed highest expression level during biofilm development was *flaA* gene. The proteome of *L. monocytogenes* was greatly influenced by biofilm development as 42 proteins were differentially synthesized in *L. monocytogenes* biofilms versus planktonic cells, and 4 of these were previously reported to relate to biofilm formation.

It is now apparent that microorganisms undergo significant changes during the transition from planktonic to biofilm growth. Therefore, in section 3 of chapter 2, the global gene expression of planktonic culture and biofilm

culture was determined using high-throughput RNA-seq by pyrosequencing technique. The PCA result revealed that both 24h- and 168h-aged biofilm were distinct from each other and as well as from planktonic stage culture suggesting that bacterial cells inside biofilm exhibited lifestyle completely different from planktonic cells. Gene expression pattern of two biofilm conditions toward planktonic culture indicated diversity of genetic factors participating in *L. monocytogenes* biofilm formation. Genes exhibited overexpression in biofilm culture (>2 $\log_2$ differential expression comparing to planktonic culture) appeared to link to flagella and motility, cell envelope biosynthesis, energy generation and intermediary metabolism, and stress response mechanisms Besides, large group of unknown function genes show up-regulation in biofilm culture suggesting that new aspects of bacterial biology are probably expressed during biofilm formation.

Due to the fact that biofilm naturally formed in environment is rather than monoculture, but the mixed-culture of various organisms. In chapter 3, the diversity and composition of bacterial microflora in food processing lines were studied together with indentifying predominant group and concerned pathogen using DGGE and pyrosequencing techniques. To begin with, bacterial species in biofilm communities in chicken processing lines were screened using DGGE. The results revealed composition of bacterial species in biofilm communities in areas that are difficult to access for cleaning, and may consequently have a risk of biofilm forming. The DGGE profile results suggest two genera of *Pseudomonas* and *Undibacterium* which are ubiquitous in processing environment both before and after cleaning, as major populations and potential biofilm-forming bacteria in these processing areas. Swab samples collected before cleaning from direct food-contacted surfaces (zone 1) and almost all from indirect food-contacted surfaces (zone 2) appear to have an identical bacterial composition. In contrast to the results observed after cleaning, all samples showed different DGGE profiles due to the fact that all bacteria could not be completely eliminated.(Section 1 of chapter 3)

Next, to overcome the limitation of DGGE, pyrosequencing technique was applied to assess the microbial community in food processing environment. The results obtained from this high-throughput method provide access to far more microflora diversity than has been ever viewed by cultivation method. The unculturable species that were ignored when using cultivation method or minor populations that were limited to detect by culture-independent method such as denaturing gradient gel electrophoresis (DGGE) could be determined in this study. Analysis results showed different characteristic of predominant microflora related to physical condition at each area. The overall microflora community structure revealed similar bacterial composition in the same zone, suggesting that bacterial community profile vary depending on things coming to contact with the surface, and also different cleaning procedure resulted in different persistent bacteria in each zone. Finally, the result of this study had added new information that the group of bacteria ubiquitous and dominate in all areas of food processing environment was thermophilic bactria and human flora.

In conclusion, overall data obtained from this research will be the fundamental for improving and implementing proper management in food plant, as the whole bacteria community should be considered in the assessment of food handling, heat treatment and sanitation concepts. Moreover, understanding the role of the specific genes and proteins during the biofilm development should permit a better understanding of the mechanisms sustaining the proliferation and the resistance of bacteria on abiotic surfaces; therefore, leading to effective control over the safety and quality of food products.